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Low Genetic Diversity and High Genetic Divergence in Southern Rear Edge Populations of *Dryas octopetala* in the High Mountains of Far East Asia

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Southern rear edge populations of cold-adapted plants at low latitudinal margins will probably be critical for determining species' responses to climate change. We focused on southern rear edge populations of an arctic-alpine plant, *Dryas octopetala*, in the high mountains of the Far East to assess genetic diversity and genetic structure by using allozyme variation and chloroplast DNA sequences. Allozyme data revealed low genetic diversity within populations (H_e = 0.003–0.144), but high genetic differentiation among populations (G_{ST} = 0.403), indicating characteristics of small and isolated populations that have persisted on mountain sky islands. The loss of genetic diversity was particularly pronounced in central Japan. On the basis of the cpDNA-haplotype distribution, the distinctive relationship between central Japan and not only northern Japan but also parts of Eurasia suggests that southernmost populations in the high mountains of central Japan are long-standing relicts of the Quaternary climate oscillations. Their refugial history therefore shaped the genetic diversity and genetic structure of the southern rear edge populations in Far East Asia.

Keywords: allozymes, arctic-alpine plant, chloroplast DNA, genetic differentiation, genetic diversity

Many arctic-alpine plants, so-called 'circumpolar plants', have vast ranges in the boreal belt of Eurasia and North America. Whereas the present distribution of arctic-alpine plants is beyond or above the potential tree line, fossil records indicate that arctic-alpine species spread southward into unglaciated areas to survive during Late-Quaternary glacial periods (Birks 2008). Since the last ice age, arctic-alpine plants have been migrating from their refugia upward to higher altitudes or northward to higher latitudes. Thus, the distribution of arctic-alpine plants has dramatically changed in response to Quaternary climate oscillations, causing them to experience frequent range expansions and fragmentations (Abbott & Brochmann 2003, Abbott 2008). In fact, many phylogeographic studies have revealed the genetic imprint of climate shifts in arctic-alpine species (e.g., Abbott *et al*. 2000, Skrede *et al*. 2006, Ikeda *et al*. 2008, 2009, Hirao *et al*. 2011, Winkler *et al*. 2012, Eidesen *et al*. 2013).

The genetic imprint of range shifts are remarkable in marginal populations. For arctic-alpine species, populations on their northern margin represent the expanding edges that colonize ice-free areas following glacial retreat (e.g., Alsos *et al.* 2007), whereas the southern marginal populations represent the rear edges that have persisted or become extirpated (e.g., Chung *et al.* 2013). In contrast to the northern expanding populations, which are newcomers, the southern rear populations are old and disproportionately significant in terms of both evolution and conservation (Hampe & Petit 2005). Southern rear edge populations of arctic-alpine species are isolated remnants of a more widespread distribution that existed during the Quaternary cold stages, and are therefore called “glacial relicts” (Schmitt *et al.* 2010, Hampe & Jump 2011). Southern relict populations have survived in the present interglacial refugia and even in some cases in long-standing refugia across glacial and interglacial periods. In addition, southern relict populations are typically small and isolated from the northern continuous, core populations. Their small size and prolonged isolation may possibly result in low levels of genetic diversity within a population and high levels of genetic differentiation among populations, thereby preserving high genetic distinctiveness. A survey of the population genetics of southern rear edge populations of arctic-alpine species may provide insights into the genetic consequences of historical isolation and fragmentation within plant species.

In this study, intraspecific genetic diversity of southern rear edge populations of an arctic-alpine species, *Dryas octopetala* L. (Rosaceae), was investigated to test the above hypotheses. *Dryas octopetala* is a long-lived shrub (life expectancy of more than 500 years; De Witte *et al.* 2012) and an important component of the arctic tundra and alpine heath in the Northern Hemisphere. Individuals of *D. octopetala* form cushions by clonal proliferation. The flowers are self-fertile (Molau 1993, Wada 1999), and the fruits are wind-dispersed achenes with elongated, feathery styles. *Dryas octopetala* is widely distributed from the Eurasian and American arctic tundra to temperate mountains of Europe, North America, and Asia (Hultén 1959, 1968, Hultén & Fries 1986). Although *Dryas octopetala* shows greatest abundance at high latitudes, usually considered to be its core range, the southern populations at low latitudes are small and isolated. In a previous phylogeographic study on *D. octopetala sensu lato* throughout most of its range, Skrede *et al.* (2006) found intrapopulation genetic diversity to be greater in northern populations than in southern populations. That study did not include samples from Far East Asia, however, where the intraspecific taxon, *D. octopetala* var. *asiatica* (Nakai) Nakai (Nakai 1932), occurs. The Asian endemic variety *asiatica* has more robust stems and shorter, broader, and blunter leaves than variety *octopetala* L. (Nakai 1932). Variety *asiatica* occurs in the high mountains of northeastern China (northern part of the Korean Peninsula) and in the Japanese Archipelago, where some of the southernmost populations are found. We therefore focused on southern rear edge populations *D. octopetala* var. *asiatica* in the high mountains of Far East Asia to assess their genetic diversity and genetic structure.

The unique phylogeographic history of arctic-alpine plants in Far East Asia compared with Europe has been documented by, e.g., Ikeda *et al.* (2006). In the European Alps, arctic-alpine species survived glaciations during the Pleistocene ice ages either in nunatak refugia (e.g., Westergaard *et al.* 2011) or in peripheral-nonglaciated refugia (Brochmann *et al.* 2003, Alsos *et al.* 2007). In contrast, Far East Asia remained free of a continuous cover of solid ice during the Pleistocene (Frenzel 1968, Svendsen *et al.* 2004), and high-altitude refugia have been crucial during warmer periods (interglacials) for survival against the upward encroachment of forest species. In fact, previous phylogeographic surveys revealed that the high mountains in the central region of mainland Japan have been long-term refugia for a variety of alpine plant species (reviewed in Fujii & Senni 2006) and have harbored unique haplotypes (e.g., Ikeda *et al.* 2008). Therefore, the unique refugial history should influence genetic diversity in rear edge populations of arctic-alpine species in Far East Asia.

TABLE 1. Overview of the collected materials of *Dryas octopetala*.

ID	Population	Region	Latitude/ Longitude	Altitude (m)	Year	Collector(s)*	n_1	n_2	Chroloplast DNA haplotype
P1	Warusawadake	Central Honshu, Japan	35°27’N/ 138°09’E	3000	2005	TM, MT	30	12	A(12)
P2	Kisokomagatake	Central Honshu, Japan	35°47’N/ 137°49’E	2800	2005	NW, AH	30	10	A(10)
P3	Yatsugatake	Central Honshu, Japan	35°58’N/ 138°22’E	2760	2005	NW, MM	30	10	A(10)
P4	Suishodake	Central Honshu, Japan	36°25’N/ 137°36’E	2930	2003	NW, RM	30	-	-
P5	Tateyama	Central Honshu, Japan	36°34’N/ 137°37’E	2700	2003	NW, RM	60	-	-
P6	Yukikuradake	Central Honshu, Japan	36°47’N/ 137°45’E	2580	2005	NW, AH	30	10	A(10)
P7	Taisetsuzan	Hokkaido, Japan	43°40’N/ 142°55’E	2060	2006	NW, KM	30	12	B(12)
P8	Changbaishan	Northeast China	42°02’N/ 128°04’E	2260	2004	NW, KK, QJL, XL	30	12	C(12)
P9	Hemavan	Scandinavian Peninsula, Sweden	65°52’N/ 15°14’E	840-860	2009	AS	-	10	F(3), G(5), H(1), I(1)

n_1 and n_2 are the number of individuals used in allozyme analysis and chroloplast DNA analysis, respectively.
*Collectors: TM Takehiro Masuzawa, MT Miki Tomita, NW Naoya Wada, AH Akira Hirao, MM Madoka Miyazaki, RM Rie Matsuzato, KM Katsutoshi Matsunaga, KK Kunio Kawada, QJL Qi-Jing Liu, XL Xuefeng Li, AS Ayako Shimono.

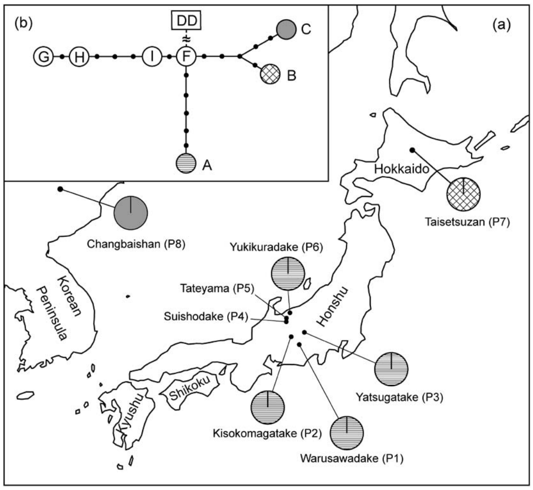

FIG. 1. (a) Sampling locations of the eight populations of *Dryas octopetala* var. *asiatica* and a summary of cpDNA haplotypes. Population localities are summarized in Table 1. (b) A median-joining network of the seven cpDNA haplotypes detected in *D. octopetala*, including four haplotypes (F, G, H, I) from Sweden in northern Europe (see Table 1 for details). Small filled circles indicate missing haplotypes. Abbreviation: DD = *Dryas drummondii*.

In this study, we surveyed the levels and distribution of genetic diversity in *Dryas octopetala* var. *asiatica*, ranging in origin from northeastern China to the Japanese Archipelago, using 19 allozyme loci and sequences of two chloroplast DNA (cpDNA) regions. We tested our predictions of low genetic diversity and high genetic divergence in the southern rear edge populations on mid-latitude mountains of the Far East, consistent with the characteristics of climate relicts (Hampe & Jump 2011).

Materials and Methods

Sampling

We sampled fresh leaves of *Dryas octopetala* var. *asiatica* from six populations in central Honshu (mainland Japan), including the southernmost population on Warusawadake, one population in Hokkaido (northern Japan), and one population in northeastern China, for both allozyme and cpDNA analyses (Table 1, Fig.1a). All populations were established on fellfields on wind-blown ridges where ericaceous dwarf shrubs, such as *Vaccinium uliginosum* L., were relatively common and where winter ground temperatures were approximately −15°C to −20°C (Wada *et al.* 2006, Wada 2009). Fresh leaves from 30 to 60 plants within each population were sampled. The collections were from plants spaced at least 2 m apart to ensure selection from a broad area. The collected leaves were transported in a cooler and stored at −78°C prior to the experiments.

Additionally, we obtained 10 specimens of *Dryas octopetala* var. *octopetala* from Hemavan, Sweden, for cpDNA sequence analysis (see details in Table 1). The voucher specimens from Sweden were deposited in the herbarium of the Sugadaira Montane Research Center at the University of Tsukuba. Moreover, three samples of *D. drummondii* Rick. from Kananaskis, Canada, were used as an outgroup in the phylogenetic analyses.

Allozyme variations

The leaf tissue was homogenized in 1.0 mL of cold extraction buffer containing 0.1 mM Tris-HCl, 1 mM EDTA (4Na), 10 mM KCl, 10 mM $MgCl_2$, 0.4% 2-mercaptoethanol, and 10% polyvinylpolypyrrolidone with the pH adjusted to 7.5. Enzymes were resolved on 7.5% polyacrylamide gels following the procedures of Shiraishi (1988). We analyzed the following 13 enzymes: aspartate aminotransferase (AAT; 2.6.1.1; three loci), acid phosphatase (ACP; 3.1.3.2; one locus), alcohol dehydrogenase (ADH; 1.1.1.1.; two loci), diaphorase (DIA; 1.6.4.1; two loci), glucose-6-phosphate dehydrogenase (G6PD; 1.1.1.49; one locus), isocitrate dehydrogenase (IDH; 1.1.1.42; one locus), leucine aminopeptidase (LAP; 3.4.11.1; two loci), malate dehydrogenase (MDH; 1.1.1.37; one locus), phosphoglucose isomerase (PGI; 5.3.1.9; one locus), phosphoglucomutase (PGM; 5.4.2.2; two loci), 6-phosphogluconate dehydrogenase (6PGD; 1.1.1.44; one locus), shikimate dehydrogenase (SKD; 1.1.1.25; one locus), and triosephosphate isomerase (TPI; 5.3.1.1; one locus). We resolved the putative loci under the assumption of Mendelian inheritance because *Dryas octopetala sensu lato* is uniformly diploid with $2n = 18$ (Elkington 1971).

The allele frequencies in each population were calculated from the 19 loci encoding the 13 enzyme systems. A locus was considered polymorphic if the most common allele had a frequency less than 0.99. We calculated standard measures of genetic variation, including the percentage of polymorphic loci (P), mean number of alleles per locus (A), and expected heterozygosity or gene diversity (H_e). Deviations from Hardy-Weinberg expectations were quantified for each polymorphic locus within each population with Wright's fixation index (F). Fixation indices were tested for significance with the chi-squared analysis (Li & Horvitz 1953). The amount of genetic differentiation among populations at the gene pool level was estimated using the G_{ST} measure (Nei 1973) in which gene diversity across all populations (H_T) was calculated for each locus and then averaged over all loci (Culley *et al*. 2002). To visualize similarities between populations, we performed a principal coordinate analysis (PCO) based on Nei's standard genetic distance with sample size bias correction (Nei 1978) using the R software package (R Core Team 2013). The relationships between genetic and geographic distance (isolation by distance) among all populations were estimated (Slatkin 1987, 1993, Rousset 1997) by correlating $G_{ST}/(1-G_{ST})$ with the natural logarithm of geographic distance (km) in a Mantel test with 9999 permutations using the R software package.

Sequence of chloroplast DNA

Two non-coding cpDNA regions were sequenced: the *atpB-rbcL* spacer (Hodges & Arnold 1994) and the *trnV-ndhC* spacer (Shaw *et al*. 2007). We analyzed 10 to 12 plants per population, with the exception of the Tateyama and Suishodake populations, from which every sample was used for the prior allozyme analysis. DNA was extracted by the cetyltrimethyl-ammonium bromide (CTAB) method (Stewart Jr & Via 1993) or by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. PCR amplification was performed in a 10-µL reaction mixture containing 0.5 µL of template DNA, 5 µl of 2× AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA, USA), and each pair of primers at 0.2 µM: *atpB* and *rbcL* (Terachi 1993), and *trnV* $^{(UAC)}$x2 and *ndhC* (Shaw *et al*. 2007). The amplification parameters were 95°C for 10 min; 35 cycles at 95°C for 1 min, 50°C for 1 min with a ramp of 0.3°C/s, and 65°C for 4 min; and 65°C for 5 min following the "*rpl16*" cycling program (Shaw *et al*. 2005). The PCR products were purified using an ExoSAP-IT Kit 0.1× (GE Healthcare, Little Chalfont, UK). The cycle sequencing reactions of forward and reverse strands were performed with the same PCR primers, using 0.1× diluted BigDye v.3.1 (Applied Biosystems). The sequencing products were analyzed on an ABI Prism 3130 automated sequencer (Applied Biosystems). Forward and reverse sequences were assembled using BioLign (Hall 2001). The DNA sequences were aligned using MEGA6 (Tamura *et al*. 2013) and the multiple sequence alignment was refined manually. Indels were treated as single characters resulting from one mutation event. Haplotype was determined based on a combination of the two noncoding cpDNA regions. Haplotype diversity (h) and nucleotide diversity (π) were calculated at the population level using the program DnaSP version 5.0 (Librado & Rozas 2009). A median-joining network with *Dryas drummondii* as an outgroup was inferred using NETWORK v. 4.6.0.0 (Bandelt *et al*. 1999), with the indels coded as a fifth state.

Results

Allozyme diversity

Nineteen loci (*AAT-1, AAT-2, AAT-3, ACP, ADH-1, ADH-2, DIA-1, DIA-2, G6PD, IDH, LAP-1, LAP-2, MDH, PGI, PGM-1, PGM-2, 6PGD, SKD*, and *TPI*), coding for 13 enzymes, were resolved (Appendix I). Seven loci (*ACP, DIA-2, IDH, LAP-1, MDH, PGM-1*, and *TPI*) were monomorphic, whereas 12 loci (*AAT-1, AAT-2, AAT-3, ADH-1, ADH-2, DIA-1, G6PD, LAP-2, PGI, PGM-2, 6PGD*, and *SKD*) showed considerable polymorphism in at least one of the populations. Most alleles were shared among populations, but 12 private alleles (*sensu* Slatkin 1985) were present (Table 2, Appendix I). Ten of the 12 private alleles were detected on Changbaishan, one was detected on Taisetsuzan, and another one was detected on Yatsugatake.

Southern rear edge populations of *Dryas octopetala* var. *asiatica* showed low levels of allozyme diversity that varied substantially among individual populations (Table 2). The mean percentage of polymorphic loci (P) across all populations was 0.684, with values at the population level ranging from 0.105 to 0.579 with a mean of 0.224. Overall, the mean number of alleles per locus (A) was 1.9, and the estimates at the population level ranged from 1.1 to 1.7 with a mean of 1.2. The observed heterozygosity (H_O) ranged from 0.011 to 0.160 with a mean of 0.042. Although locus-specific significant deviations from Hardy-Weinberg expectations were found (four positive, two negative) among 26 tests, the mean fixation index (F) of each population was not significant (Appendix II). The gene diversity across all populations (H_T) was 0.070, whereas the values at the population level (H_e) ranged from 0.003 to 0.144 with a mean of 0.042. The population at Changbaishan in northeastern China had the highest level of genetic diversity (0.144) while populations in the Japanese Archipelago exhibited H_e values (0.003–0.068) less than half of that (Table 2). Within the populations from the Japanese Archipelago, the population on Yatsugatake (P3) had the highest level of genetic diversity. The estimated genetic differentiation among populations, G_{ST}, was 0.403, indicating that approximately 40% of the total genetic diversity resulted from differences among populations.

The genetic similarities between populations were analyzed by means of PCO (Fig. 2). The first component (PCO1), which accounted for 73.2% of the variation, separated the populations in Japan (P1–P7) from the Changbaishan population in northeast China (P8). The second component (PCO2), which accounted for 14.1% of the variance, separated the Taisetsuzan population in northern Japan (P7) from the remainder of the populations. In the scatter plot, we identified a cluster of six populations in central Japan (P1–P6) that were situated between the northern Japan population (P7) and the northeastern China population (P8). Within populations in central Japan (P1–P6), however, the Yatsugatake population (P3) was plotted at a distance from the remaining five populations. Population differentiation followed a general pattern of isolation by dis-

TABLE 2. Allozyme diversity of the eight populations of *Dryas octopetala* var. *asiatica* in Far East Asia.

ID	Population	P	A	A_{private}	H_O	H_e
P1	Warusawadake	0.211	1.2	0	0.047	0.048
P2	Kisokomagatake	0.158	1.1	0	0.021	0.026
P3	Yatsugatake	0.316	1.3	1	0.053	0.068
P4	Suishodake	0.105	1.1	0	0.004	0.003
P5	Tateyama	0.158	1.1	0	0.013	0.012
P6	Yukikuradake	0.105	1.1	0	0.011	0.009
P7	Taisetsuzan	0.158	1.1	1	0.025	0.024
P8	Changbaishan	0.579	1.7	10	0.160	0.144
	Average	0.224	1.2	1.5	0.042	0.042
	Overall	0.684	1.9	12	0.042	0.070

P is proportion of polymorphic loci, A is the mean number of allele per locus, A_{private} is the number of “private allele” (Slatkin1985), H_O is the observed heterozygosity, and He is the expected heterozygosity or gene diversity.

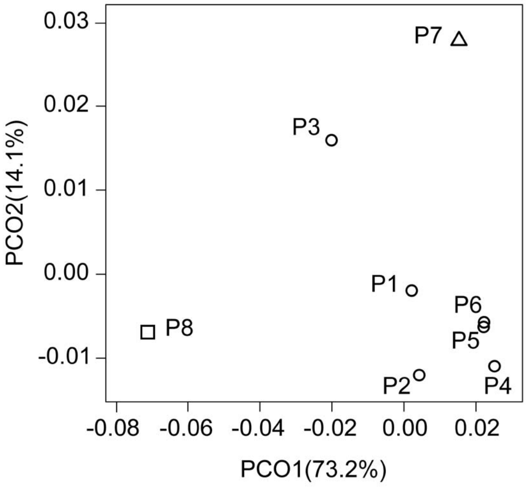

FIG.2. Principal coordinate analysis of the eight populations of *Dryas octopetala* var. *asiatica* based on Nei’s genetic distance obtained from allozyme analysis. Circles, the triangle, and the square show the populations in central Japan (P1-P6), the population in northern Japan (P7), and the population in northeast China (P8), respectively. See Table 1 and Figure 1 for details regarding the populations.

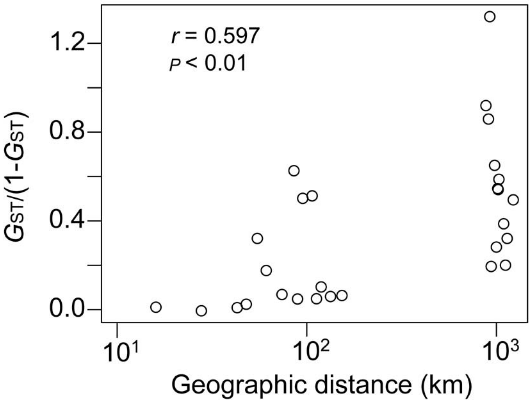

FIG.3. Pattern of isolation by distance for all pairs of the eight populations of *Dryas octopetala* var. *asiatica*.

tance; Mantel test showed a significant pattern over the entire geographic range (r = 0.597, p < 0.01: Fig. 3).

cpDNA haplotypes and their geographic distribution

The concatenated sequence of the two noncoding regions of cpDNA had a length of 1173 bp, of which 13 sites were variable with five substitutions and eight indels. Three haplotypes (A, B, and C) were identified across 64 samples of *Dryas octopetala* var. *asiatica* from Far East Asia (Table 1, Fig. 1). Additionally, four haplotypes (F, G, H, and I) were detected in samples from Sweden in northern Europe. The sequences are available at DDBJ/EMBL/GenBank (AB685700–AB685705, AB720971, AB720972, and AB720975–AB720977). The median-joining network revealed three distinct clades (Fig. 1b). One specific clade contained only the single haplotype (A) from central Japan. The second clade, comprising haplotypes B and C, occurred in northern Japan and northeast China, respectively. Finally, an additional clade, which included haplotype F, G, H, and I, was found in Sweden. Haplotype F was located in the central part of the network. All populations in Far East Asia were fixed by a single haplotype, whereas the population in Sweden was genetically divergent (haplotype diversity, $h = 0.711$; nucleotide diversity, $\pi = 0.00048$).

Discussion

The geographic distribution of the cpDNA haplotypes in Far East Asia indicates an extensive genetic structure among central Japan, northern Japan, and northeast China, in each of which a unique haplotype was fixed. All populations in central Japan were of cpDNA haplotype A, which is phylogenetically distinct not only from haplotype B in northern Japan but also from haplotype C in northeast China (Fig. 1). This result is consistent with previous phylogeographic studies of Japanese alpine plants, which hypothesized that the high mountains of central Japan served as long-standing isolated refugia during Quaternary climatic oscillations (reviewed in Fujii & Senni 2006). Thus, the southernmost populations of central Japan should be older relicts that have survived more than one glacial/ interglacial cycle. Altitudinal shifts in mountains over 3000 m a.s.l. in central Japan may allow long-term population persistence of arctic-alpine plants, some of which have speciated *in situ* (Ikeda *et al*. 2012, 2014). In Far East Asia, moreover, the lack of extensive glaciation (Frenzel 1968, Svendsen *et al*. 2004) has contributed to the presence of long-standing refugia. In Europe, however, southern alpine populations have a different evolutionary history because glaciers obliterated most of their previous areas of distribution. Although some phylogeographic studies have referred to glacial relicts of arctic-alpine species in the Alps (e.g., Reisch *et al*. 2003), a survey of the literature revealed that most phylogeographic patterns of arctic-alpine species in Europe are the result of recent (in most cases postglacial) vicariance or dispersal events (Schmitt *et al*. 2010). Therefore, the long-standing refugia in central Japan provide a unique phylogeographic history along with the impetus for shaping the genetic diversity and genetic structure of the southern rear edge populations in Far East Asia.

Allozyme data revealed low genetic diversity in *Dryas octopetala* var. *asiatica* within the overall population ($H_T = 0.070$) and at the population level ($H_e = 0.003–0.144$) (Table 2). Of particular note is that the level of genetic diversity in southern populations was much lower than has been reported for more northern populations around the North Atlantic ($H_e = 0.057–0.423$; Philipp & Siegismund 2003) and for other boreal-temperate and woody plants ($H_e = 0.272$ and 0.298; Hamrick & Godt 1989). The low genetic diversity in *D. octopetala* var. *asiatica* is supported by the cpDNA data, although based on limited sampling ($h = 0$, $\pi = 0$ in Asian Far East populations, and $h = 0.711$, $\pi = 0.00048$ in a Sweden population).

In contrast to the low level of genetic diversity within populations, high genetic differentiation was found among populations of Far East Asia ($G_{ST} = 0.403$). As supported by the cpDNA findings, allozyme divergence among the populations has increased in rear edge populations in the Far East, in contrast to northern populations of the same species: $F_{ST} = 0.00–0.11$ in Alaska (Max *et al.* 1999); ϕ_{ST} (F_{ST} analogue by AMOVA) = 0.216 in countries around the North Atlantic (Vik *et al.* 2010). Additionally, we found some evidence of isolation by distance based on allozyme analysis (Fig. 2), indicating that historical gene flow has been restricted by geographical distance but was not obliterated across Far East Asia, where topographical barriers provided by mountains and/or the sea may explain the limitation of gene flow. The southern rear edge populations of *Dryas octopetala* are located on montane sky islands on true islands in the Far East. The spatial isolation of populations inhabiting mountain sky islands enforces genetic differentiation among populations (DeChaine & Martin 2005). Geographically, northeastern China is part of the Eurasian continent, whereas the Japanese Archipelago is an island chain located along the East Asian littoral region, where populations are hypothesized to have been isolated from those on the continent. The PCO analysis, in which the first component separated the Japanese populations from the Chinese population, and the second component, which separated northern Japan from central Japan, supports isolation by sea. Geographic features serving as barriers thereby accelerate genetic differentiation among populations.

The low levels of genetic diversity were particularly pronounced in the populations of central Japan, whereas the population on Changbaishan in northeast China retained considerable genetic diversity (Table 2). The levels of within-population genetic diversity appear to be correlated with population size. Most of the populations in central Japan are small. For example, approximately 150 patches of *Dryas octopetala* var. *asiatica* occur on Tateyama (Fig. 1) (Wada 2008). The populations on Yatsugatake are exceptional in comprising relatively many patches along the main ridge from Akadake to Yokodake (A. S. Hirao & N. Wada, personal observation) and have a higher level of genetic diversity than do other populations in central Japan (Table 2). On Changbaishan in northeast China, where *Dryas octopetala* var. *asiatica* occurs from the sub-alpine to the alpine zone, the population is considerably larger. Relatively highly polymorphic loci and most of the private alleles were found in population from northeast China. Additional samples, especially outside of the Japanese Archipelago, are necessary to further clarify the trends in genetic diversity of the Asian endemic variety *asiatica*.

The reproductive system of plants has long been hypothesized to influence their genetic diversity (Hamrick & Godt 1989, Nybom & Bartish 2000). In fact, the difference in genetic diversity in two arctic-alpine plants, *Diapensia lapponica* L. and *Empetrum nigrum* L., at the southernmost edge of their range is mainly attributable to different life histories and reproductive traits (Chung *et al.* 2013). Thus, the reproductive system of *D. octopetala*, such as self-fertility (Molau 1993, Wada 1999), may also affect genetic variation. Accelerated inbreeding with decreasing population size leads to an increase in homozygocity, genetic drift, and random changes in allele frequencies. In turn, if the Japanese populations self pollinate and the Changbaishan populations outcross, trends in the level of genetic diversity may occur. We did not observe the predicted pattern in the inbreeding coefficient (Appendix II), however, possibly because of the extremely low polymorphism within the Japanese populations in which most alleles are fixed. Application of highly polymorphic markers, such as microsatellites, can identify the relationship between inbreeding and genetic diversity in southern rear edge populations.

Changes in climate threaten many species, particularly those in arctic-alpine regions (IPCC 2007, Engler *et al.* 2011, Pauli *et al.* 2012). Even within arctic-alpine species with wide-ranging distributions, there is a high risk of losing southern populations due to climate warming (Thuiller *et al.* 2005). In fact, the fossil record shows that rear edge populations are often extirpated (Davis & Shaw 2001). Furthermore, the southern populations may be genetically vulnerable, thus their viability may be overestimated. Low levels of genetic diversity at the rear edge of species' ranges may limit evolutionary potential in the context of climate change. Further clarification of the evolutionary history of *Dryas octopetala* var. *asiatica* can improve our understanding of the complicated response of arctic-alpine species to historical and contemporary climate change.

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APPENDIX I. Allele frequencies at 19 allozyme loci in the eight populations of *Dryas octopetala* var. *asiatica* in Far East Asia.

Locus	Allele	Population								
		P1	P2	P3	P4	P5	P6	P7	P8	Over-all
AAT-1	*a*								0.183	0.023
	b	1.000	1.000	1.000	1.000	1.000	1.000	0.867	0.817	0.960
	c							0.133		0.017
AAT-2	*a*	1.000	1.000	0.833	1.000	1.000	1.000	1.000	1.000	0.979
	b			0.167						0.021
AAT-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833	0.979
	b								0.150	0.019
	c								0.017	0.002
ACP	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADH-1	*a*	0.817	0.800	0.450	1.000	1.000	1.000	1.000	0.050	0.765
	b	0.183	0.200	0.550					0.950	0.235
ADH-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.983	0.998
	b								0.017	0.002
DIA-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.617	0.952
	b								0.383	0.048
DIA-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G6PD	*a*	0.283	0.100	0.767	0.033	0.100	0.100	0.867	0.483	0.342
	b	0.717	0.900	0.233	0.967	0.900	0.900	0.133	0.517	0.658
IDH	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2	*a*								0.033	0.004
	b			0.017					0.217	0.029
	c	1.000	1.000	0.983	1.000	1.000	1.000	1.000	0.717	0.963
	d								0.033	0.004
MDH	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGI	*a*								0.017	0.002
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.983	0.998
PGM-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-2	*a*	1.000	1.000	0.933	1.000	1.000	1.000	1.000	1.000	0.992
	b			0.067						0.008
6PGD	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.433	0.929
	b								0.567	0.071
SKD	*a*								0.017	0.002
	b	0.117				0.025			0.033	0.022
	c	0.883	1.000	1.000	1.000	0.975	1.000	1.000	0.950	0.976
TPI	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

APPENDIX II. Wright's inbreeding coefficient, *F*, for polymorphic loci in the eight populations of *Dryas octopetala*. *Associated genotype frequencies deviate significantly from Hardy-Weinberg expectations (chi-square test, $p < 0.05$).

Locus	Population							
	P1	P2	P3	P4	P5	P6	P7	P8
AAT-1	—	—	—	—	—	—	-0.154	-0.002
AAT-2	—	—	-0.200	—	—	—	—	—
AAT-3	—	—	—	—	—	—	—	0.057
ACP	—	—	—	—	—	—	—	—
ADH-1	0.666*	0.375*	0.529*	—	—	—	—	0.649*
ADH-2	—	—	—	—	—	—	—	-0.017
DIA-1	—	—	—	—	—	—	—	-0.481*
DIA-2	—	—	—	—	—	—	—	—
G6PD	-0.395*	-0.111	0.255	-0.034	-0.111	-0.111	0.135	-0.068
IDH	—	—	—	—	—	—	—	—
LAP-1	—	—	—	—	—	—	—	—
LAP-2	—	—	-0.017	—	—	—	—	0.009
MDH	—	—	—	—	—	—	—	—
PGI	—	—	—	—	—	—	—	-0.017
PGM-1	—	—	—	—	—	—	—	—
PGM-2	—	—	-0.071	—	—	—	—	—
6PGD	—	—	—	—	—	—	—	-0.086
SKD	-0.123	—	—	—	-0.026	—	—	-0.040
TPI	—	—	—	—	—	—	—	—
Mean	0.013	0.200	0.223	-0.034	-0.093	-0.111	-0.010	-0.107